ON THE RESISTANCE OF ASCARIS EGGS

SADAO YOSHIDA

Pathological Department, Osaka Medical College, Osaka, Japan

For several years, in my study on the development of *Ascaris lumbricoides,* I have been testing the resistant power of ascarid eggs against various chemical media in which the eggs were cultured. As far as references go, there are few reports on this subject, notwithstanding it is very important for prevention of ascaris infection.

Method: In the earlier part of my investigation I collected the eggs from the patient's feces dissolved in water, by filtering and then by centrifuging. The collected eggs are treated with a reagent in which the eggs are to be cultured. In the later part, however, I put the fecal mass in the reagent for some time, longer or shorter according to the purpose of experiments, then the eggs were collected from the fecal solution of the reagent by filtering and centrifuging, and lastly were put in a culture dish of the same concentration of reagent as that in which the above treatment is performed.

Culture dishes were kept in the laboratory room in the summer months and in the incubator at 31° C. in the winter.

The vitality of eggs and embryos in each dish was examined at regular intervals of time. The distinction between dead or living eggs was decided partly by microscopical examination and partly by animal feeding experiments. Young or immature eggs were chiefly tested by microscopical observation. The vital power was observed in some cases by transferring the eggs from a reagent into a water culture to test the further development. Even in the same culture there are a great many individual differences in longevity as in other organisms. Thus in the following tables the word "dead" means that the majority of the eggs in the culture indicated are dead, while there are very few eggs alive; and the word "alive" shows a majority of living eggs. Hence it is very difficult to determine exactly the date on which the eggs die or still live in a reagent.

Experiments: During the time from August, 1917, to January, 1918, I carried on a great many experiments, the result of which I published briefly in a Japanese journal of medicine. That is not so important and valuable, for it is all covered by the results obtained in the recent experiments, the results of which are tabulated as follows:

TABLE 1.—AFTER KEEPING THE FECAL MASS IN EACH REAGENT FOR TEN DAYS FROM JANUARY 11 TO 21, 1919, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C.

Reagents	Day							
	13th	18th	28th	31st	38th	43d	52d	
0.5% Nitric acid	E appear	EH normal E alive	do	do	do	do	do	Alive
1% Hydrochloric acid	E alive	do	do	do	do	do	do	Alive
5% Hydrochloric acid	E alive	EH swollen E alive	EH absent present E alive	do	do	do	do	Alive
7% Sulphuric acid	EH absent present E appear	E alive	EH absent or thinned E alive	E alive	do	E shrink		Dead
7% Glacial acetic acid	EH swollen E alive	EH destroyed E alive	EH dest. or absent E alive	E do	do	do	do	Alive
10% Formalin	EH normal E alive	do	E alive	do	do	do	do	Alive
12.5% Formalin	E alive	do	dried up					

E, embryo, EH, albuminous coating.

This table shows that the eggs in 7 per cent. sulphuric acid develop into embryos, but sooner or later they all die. On the twenty-eighth day (February 18) a part of each culture was transferred into the water culture, the further development of which is stated in Table 6.

TABLE 2.—AFTER FOUR HOURS IMMERSION OF FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C., ON FERUARY 3

Reagents	Day							
	5th	11th	15th	18th	25th	36th	49th	
0.5% Carbolic acid	F no or rarely 2	EH normal F no	F 2-4 V appear	V	do	do	do	Dead
1% Nitric acid	EH destroyed or absent F many	E appear	EH absent E alive	E alive	do	do	do	Alive
10% Hydrochloric acid	EH normal F many	EH swollen E alive	do	E alive F vacuol	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do V appear	E appear	E shrunk	do	E V	do	Dead
10% Glacial acetic acid	EH absent F many	do E alive	E alive F many	do	E alive V appear	E V	do	Dead
15% Formalin	EH normal F many	do V	F V	do	E few	E V	do	Dead
20% Formalin	EH normal F 6-8	.....	F 6-8 V	....	.....	..	..	Dead

F, blastomere; V, vacuole.

The table shows that in 0.5 per cent. carbolic acid or 20 per cent. formalin, ascaris eggs are unable to develop into embryos; in 10 per cent. sulphuric acid or glacial acetic acid, or 15 per cent. formalin, the eggs develop into embryos, but sooner or later die.

TABLE 3.—AFTER FIVE HOURS IMMERSION OF THE FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 10

Reagents	Day							
	4th	8th	11th	18th	23d	36th	42d	
0.6% Carbolic acid	EH normal F no	do V	V	do	do	do	do	Dead
1% Corrosive sublimate	EH normal F 4-6	F many	do	E appear	E alive F many	do	do	Alive
1.5% Nitric acid	EH absent or swollen F 2-7	do E appear	E alive	do	do	do	do	Alive
12.5% Sulphuric acid	EH normal F 2-4-6	do E appear	E shrink	do	do	do	do	Dead
12.5% Glacial acetic acid	EH swollen or absent F 2-4-6	do E appear	E alive	do	E alive V appear	E V	do	Dead
15% Hydrochloric acid	EH normal F 1-4	EH ab, or present E appear	E alive F V	do	E shrunken V appear	do	do	Dead
20% Hydrochloric acid	EH normal F 4-7	EH absent or swollen E appear F V	E alive or shrunken	E shrink and V	do	do	do	Dead
25% Formalin	EH normal F no or 2-4	do	F V appear	F V	do	do	do	Dead
Human urine	EH normal F no	do	do	rarely F 2	do	V appear	V	Dead

From this table it is seen that in 0.6 per cent. carbolic acid, or in 25 per cent. formalin, ascaris eggs are unable to develop into embryos; in 12.5 per cent. sulphuric acid or glacial acetic acid, or in 15 per cent. and 20 per cent. hydrochloric acid, the eggs may develop into embryos, but die later; 1.5 per cent. nitric acid is not harmful to the development of the eggs.

TABLE 4.—AFTER FOUR HOURS IMMERSION OF FECAL MASS, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C ON FEBRUARY 14

Reagents	Day					
	5th	14th	19th	30th	43d	
0.1% Potassium permanganate	EH normal F no	rarely do F 2-4	E alive	do	do	Alive
0.5% Potassium permanganate	EH normal F no	rarely do F 2-4	E alive	do	do	Alive
1% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
5% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do E appear	E alive or shrunken	E V	do	Dead
15% Hydrochloric acid	EH normal F many	slightly shrunken	shrunken	V	do	Dead
20% Hydrochloric acid	EH swollen destroy F many	F many V appear	V	do	do	Dead
1% Nitric acid	EH slightly swollen F many	do E appear	E alive	do	do	Alive

The embryos cultured in 0.5 per cent. solution of potassium permanganate emerged from the egg-shell alive. This is a most interesting fact in the study of ascaris development.

TABLE 5.—AFTER TWO DAYS IMMERSION OF FECAL MASS IN THE REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 20

Reagents	Day			
	8th	13th	37th	
0.02% Potassium permanganate	EH normal F no rarely 2-4	F many E appear	E alive	Alive
0.05% Potassium permanganate	EH normal F no rarely 2-4	E alive	do	Alive
10% Iron sulphate	EH normal E appear	F many E alive	E alive	Alive

TABLE 6.—AFTER TWENTY-EIGHT DAYS, THE EGGS WERE TRANSFERRED FROM THE REAGENT (TABLE 1) TO THE WATER CULTURE ON FEBRUARY 18

Date	Reagents				
	0.5% Nitric Acid	5% Hydrochloric Acid	7% Sulphuric Acid	7% Glacial Acetic Acid	10% Formalin
21st day	E alive	E alive	F many E appear	do	F many
34th day	E alive	do	do	do	F many E alive

TABLE 7.—AFTER FIFTEEN DAYS, THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE ON FEBRUARY 18 (See TABLE 2)

Date	Reagents						
	0.5% Carbolic Acid	1% Nitric Acid	10% Hydrochloric Acid	10% Sulphuric Acid	10% Glacial Acetic Acid	15% Formalin	20% Formalin
10th	F no V	E alive	E alive	F shrunk	F many E appear	F many V	V
15th	V	E alive	E alive	F shrunk V	E alive	F V	V
21st	V	E alive	E alive V appear	V	E alive	V	V
34th	V	E alive	V	V	E alive	E no F V	V
	Dead	Alive	Dead	Dead	Alive	Dead	Dead

This table shows that eggs cultured in 10 per cent. hydrochloric acid or sulphuric acid, and in 15 or 20 per cent. formalin died by the fifteenth day of cultivation.

TABLE 8.—AFTER EIGHT DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT, ON FEBRUARY 18 (SEE TABLE 3)

Date	Reagents								
	0.6% Carbolic Acid	1% Corrosive Sublimate	1.5% Nitric Acid	12.5% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Formalin	25% Formalin	Urine
10th	F no V	F many	F many E alive	F many	E appear alive	F many E appear	E alive or V	V	
15th	V	F many E alive	E alive	.....	E alive	E alive			
21st	V	E alive	E alive	F shrunk	E alive	E alive	E V	F V	
34th	V	E alive	E alive	shrunk	E alive	E alive	E shrunk	V	F no 2-4
	Dead	Alive	Alive	Dead	Alive	Alive	Dead	Dead	Alive

Eggs in 0.6 per cent. carbolic acid, in 12.5 per cent. sulphuric acid or in 20 per cent. and 25 per cent. formalin were so injured as to be unable to develop further by the eighth day.

TABLE 9.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT IN THE LABORATORY ROOM, AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	15% Hydrochloric Acid	20% Hydrochloric Acid
11th day	F many E alive	F no rarely 2-7	shrunken	V
24th day	E alive	E alive	V	V
	Alive	Alive	Dead	Dead

The eggs in 15 or 20 per cent. hydrochloric acid could not develop by the fourteenth day.

TABLE 10.—AFTER TEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT CULTURE ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	0.5% Carbolic Acid	0.6% Carbolic Acid
23d day	F no rarely 2-4	F no rarely 2-4	F no rarely 2	F no rarely 2
28th day	F many E alive	F many E alive	F no or 2	F no or 2
	Alive	Alive	Alive	Alive

Ascarid eggs retain the power to develop during ten days in 0.5 or 0.6 per cent. carbonic acid.

TABLE 11.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT ON MARCH 11

Date	Reagents							
	7% Formalin	10% Formalin	15% Formalin	20% Formalin	10% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Hydrochloric Acid
13th	E alive	E alive	E alive	E alive	F	F many	EH absent E F	R alive
18th	do	do	do	do	E alive	E appear F many	E alive	do
	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive

All above experiments are summarized in a table as follows:

Potassium permanganate	0.02% alive	0.05% alive	0.1% alive	0.5% alive	
Corrosive sublimate	1% alive				
Nitric acid	0.5% alive	1% alive	1.5% alive		
Iron sulphate	1% alive	5% alive	10% alive		
Formalin	7% alive until 14th	10% alive	15% dead after 15-18	20% dead after 15-20	25% dead before 8th
Hydrochloric acid	1% alive	5% alive	10% alive	15% dead after 11-14	20% dead after 8, 11 or 14
Glacial acetic acid	7% alive	10% alive, but dead after after 25-30th	12.5% alive until 8th, dead after 28th		
Sulphuric acid	7% alive, dead after 43d	10% dead after 11-15	12.5% dead after 8-11th		
Carbolic acid	0.5% alive 8th, dead after after 11-15	0.6% alive until 10, dead after 8-11th day			
Human urine	Vacuoles appear after 36 or 42, and dead after 70th day				

K. Hotta, a student of our college, also made elaborate experiments on the same subject under my direction during the past year from June, 1918, to May, 1919. The results of his experiments coincide essentially with those of mine, with the exception of a slight difference.

The summarized tables are given as follows:

	Reagents				
	Hydrochloric Acid	Carbolic Acid	Sulphuric Acid	Formalin	Glacial Acetic Acid
Able to develop in	14%	0.3%	9%	12%	8%
Unable to develop in	15%	0.4%	10%	....	9%

Hydrochloric acid	15% alive until 12, 13	17% alive until 12, 11	19% alive until 10	25% alive until 6, 7	28% alive until 5th
Sulphuric acid	10% alive until 12th	12% alive until 11th	15% alive until 11th	20% alive one day	25% dead within day
Formalin	20% alive until 7, 8th	25% alive 7 days	27% alive 6 days		
Carbolic acid	0.4% alive until 30th	0.6% alive 11 days	0.8% alive 10 days		

It is a most important and interesting fact that ascarid eggs are unable to develop and ultimately die in human urine. From some experiments I have assumed that the urine acts more effectively upon eggs at a higher temperature (31° C.) than in the lower (10° C.) for destroying the power of their development.

The influence of the reagent on the ascarid eggs depends upon the permeability of the coverings of the egg. In the above experiments, for instance, formalin and sulphuric acid act to coagulate the albumin-coating of ascarid eggs in consequence of which the penetration of the fluid is prevented. After long action, glacial acetic acid and nitric acid destroy or break down the albuminous membrane of the eggs, but do not penetrate easily through the inner chitinous membrane. For these reasons eggs cultured in those reagents may resist a higher concentration and survive much longer. Hydrochloric acid will also do the same thing.

Carbolic acid, however, may penetrate the egg membrane more easily and more effectively than any other reagents which I have used in the above experiments. Urine contains several kinds of ferments by which the albuminous membrane of the egg may be dissolved. Action of the ferments seems to be accelerated by increasing the temperature as stated above. Moreover, the urine concentration is so much higher than that of the egg-content that the osmotic pressure is sufficiently great to facilitate introducing the urine into the egg-shell.

Besides these experiments on chemicals, I have made some other experiments on the resistance of ascaris eggs to cold.

Exp. 1.—Mature eggs cultured in the incubator from September 28, 1917, to December 11, were put under ground and covered by a thin lawer of soil, and on May 2, 1918, the eggs were given to a guinea-pig which was surely infected.

Exp. 2.—Mature eggs cultured in the incubator from October 27, 1917 to December 22, were put on the ground out-doors, and on May 2, 1918, they were given to a guinea-pig which was also evidently infected.

Exp. 3.—Immature eggs collected on December 8, 1917, were put on the ground from December 13, 1917, to May 2, 1918, then they were transferred to the incubator and kept again in the laboratory room from June to October; next they were put in the incubator during November and December, and in the laboratory room from January to February, 1919, then finally in the incubator from March 1 to 19. On the last date the eggs were given to two guinea-pigs which were killed after a week and showed an infection.